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Conditioning on Parity in Studies of Perfluoroalkyl Acids and Time to Pregnancy: An Example from the Danish National Birth Cohort

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Background: Previous studies have investigated the associations between perfluoroalkyl acids (PFAAs) in women and time to pregnancy (TTP). Inconsistent results may be explained by differences in conditioning on parity.

Objectives: We used causal directed acyclic graphs to illustrate potential confounding related to previous pregnancies and exposure measurement error due to differences in the interpregnancy interval in pregnancy-based studies that include parous women. We exemplified the potential importance of these issues using data from the Danish National Birth Cohort.

Methods: We used discrete time survival models to estimate associations between maternal plasma PFAAs in early pregnancy and TTP in 638 nulliparous and 613 parous women.

Results: PFAA quartiles were not associated with the TTP in nulliparous women. In parous women, higher PFAA quartiles were associated with longer TTP. The strongest associations were estimated for perfluorohexane sulfonate and perfluorooctane sulfonate. PFAA concentrations were higher in women with longer interpregnancy intervals. Accounting for the interpregnancy interval attenuated the estimated associations.

Conclusions: Associations between PFAAs and TTP in parous women may be biased by confounders related to previous pregnancies and exposure measurement error. To avoid these biases, studies that include parous women may need to condition on *a*) common causes of PFAAs and the TTP in the index pregnancy, *b*) previous births (a descendant of a collider), *c*) PFAA levels or common causes of PFAA levels and the TTP in the previous pregnancy (to alleviate collider stratification bias caused by conditioning on previous births), and *d*) the interpregnancy interval (in pregnancy-based studies). Alternatives would be to restrict studies to nulliparous women or to use toxicokinetic modeling to correct exposure estimates in parous women. These recommendations may be extended to studies of other chemicals with similar toxicokinetic properties. https://doi.org/10.1289/EHP1493

Introduction

Since 2009, a number of studies have investigated associations between perfluoroalkyl acids (PFAAs) in women and fecundability, indicated by the time to pregnancy (TTP), with inconsistent results (Bach et al. 2015b, 2015a, 2016b; Buck Louis et al. 2013; Fei et al. 2009; Jørgensen et al. 2014; Vélez et al. 2015; Vestergaard et al. 2012; Whitworth et al. 2012, 2016). Only one epidemiological study showed an association between higher PFAAs and longer TTP in nulliparous women (Fei et al. 2009), whereas three other studies found no association (Bach et al. 2015a; Vestergaard et al. 2012; Whitworth et al. 2016). A few other studies demonstrated associations in parous women or pooled samples of both parous and nulliparous women (Bach et al. 2016b). Thus, differences in whether and how the studies conditioned on parity could possibly, at least partly, explain the inconsistent results (Bach et al. 2016a). Some rodent studies suggest that PFAA exposure may cause impaired fertility, potentially through endocrine disruption. However, most animal studies applied exposure levels of PFAAs several orders of magnitude higher than for background-exposed humans (Bach et al. 2016b).

In this paper, we use causal directed acyclic graphs (DAGs) to illustrate confounding by factors related to previous pregnancies as well as potential dependent measurement error of the exposure in pregnancy-based studies of parous women. Further, we evaluate data from the Danish National Birth Cohort to exemplify these issues.

In a recent paper, Vélez et al. (2015) argued that the association between PFAA exposure and the TTP may be described as illustrated by Howards et al. (2012) using a DAG. Figure 1, which is adapted from Howards et al. (2012), assumes that both fecundability and PFAA levels will be similar in two consecutive pregnancies, but this is not plausible for the following reasons. First, fecundity (the biological capability to conceive) and fecundability (the probability of conception in each cycle) depend on various factors such as age (Axmon et al. 2006; Joffe and Li 1994; Mutsaerts et al. 2012; Olsen 1990), body mass index (BMI) (Gesink Law et al. 2007; Ramlau-Hansen et al. 2007; Wise et al. 2010), and smoking (Bolumar et al. 1996; Joffe and Li 1994; Olsen 1991). Consequently, fecundity and fecundability are not expected to be constant over time at the individual level. Second, PFAA concentrations also change over time. Many PFAAs have half-lives of a year or more (Olsen et al. 2007), and a study of 100 women reported that concentrations measured during the same trimester were correlated between consecutive pregnancies (Papadopoulou et al. 2015). However, a study of 19 women reported that PFAA concentrations generally decrease during pregnancy (Glynn et al. 2012), and, on average, PFAA concentrations are lower in parous compared to nulliparous women (Berg et al. 2014; Brantsæter et al. 2013; Papadopoulou et al. 2015), most likely due to the transfer of PFAAs to the fetus and their excretion in breast milk (Cariou et al. 2015; Kang et al. 2016; Mondal et al. 2014; Motas Guzmàn et al. 2016).

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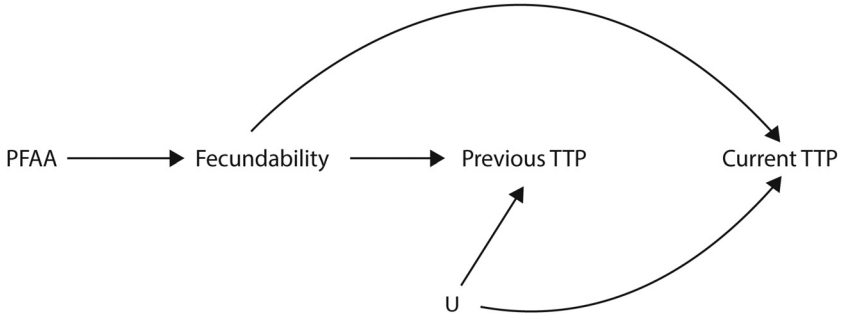

Figure 1. The association between perfluoroalkyl acids (PFAAs) and time to pregnancy (TTP) as proposed by Vélez et al. (2015) and adapted from Howards et al. (2012), Figure 1B.

Vélez et al. (2015) argued that conditioning on parity should be avoided in studies of PFAAs and TTP, and stated "In a sense, [previous TTP] is a proxy for parity and fecundability." In our opinion, however, the time-varying nature of PFAA exposures needs to be considered, and fecundability (or rather, its proxy measure, the TTP) and parity (previous births) should be treated as separate variables in causal models (Bach et al. 2015a). Consequently, we propose an alternative causal structure, shown in Figure 2. In all subsequent figures, the association of interest is the association between PFAAs at the time of starting to try to conceive and the TTP, independent of parity. For nulliparous women, this is illustrated by the association between PFAA 1 and impaired fecundity 1, and for parous women, by the association between PFAA 2 and impaired fecundity 2. Figure 2 is built on several assumptions. First, although PFAA concentrations in consecutive pregnancies are temporally distinct, PFAA concentrations in the first pregnancy affect PFAA concentrations in the second pregnancy. Second, impaired fecundity (as measured by the TTP, which is not easily expressed in a DAG) affects the chance of conception, gestation, and birth. Genetics or other unmeasured factors such as tubal factors or male factors (U) are likely to affect fecundity for both pregnancies as well as the chance of a successful gestation. Third, previous gestation and confounders related to the first pregnancy may affect the confounders for the second pregnancy. Important confounders, i.e., common causes of PFAA concentrations and the TTP, may include, for example, age (Axmon et al. 2006; Berg et al. 2014; Joffe and Li 1994; Mutsaerts et al. 2012), BMI (Gesink Law et al. 2007; Ramlau-Hansen et al. 2007; Wise et al. 2010), and socioeconomic status (Brantsæter et al. 2013; Mutsaerts et al. 2012). Finally, we assumed no causal association between PFAAs and the TTP, and thus, no arrow leads from PFAAs to the TTP in this scenario. To obtain unbiased estimates in parous women under the scenario shown in Figure 2 (in which U remains unmeasured), it is necessary to condition on previous births (a descendant of a collider) and to adjust for PFAAs in the preceding pregnancy (PFAA 1), or adjust for confounders of the association between PFAAs and fecundability in the previous pregnancy (C1), in addition to adjusting for confounders of the association between PFAAs and fecundability in the current pregnancy (C2). Note that conditioning on a descendant of a collider, i.e., previous births, is necessary in order to close other open backdoor paths, and the backdoor paths opened by conditioning on this descendant of a collider may be closed by conditioning on PFAA levels or confounders from the preceding pregnancy.

If a causal arrow is assumed from the PFAA levels in the preceding pregnancy (PFAA 1) to impaired fecundity in the preceding pregnancy (Impaired fecundity 1), it is necessary to adjust for PFAA levels from the preceding pregnancy (PFAA 1) in addition to previous births and confounders measured in relation to the current pregnancy (C2) in order to close all back doors. Of note, under this assumption, conditioning on confounders from the preceding pregnancy (C1) in addition to previous births and confounders measured in relation to the current pregnancy (C2) will not be sufficient.

Previous studies have suggested that a rationale for the restriction to nulliparous women is to avoid potential reverse causation among parous women in pregnancy-based studies in which exposures were obtained after conception (Olsen et al. 2009; Vestergaard et al. 2012; Whitworth et al. 2012). As shown in Figure 3, this issue may reflect differential exposure measurement error, given that the TTP would affect PFAA concentrations measured during pregnancy rather than PFAA concentrations

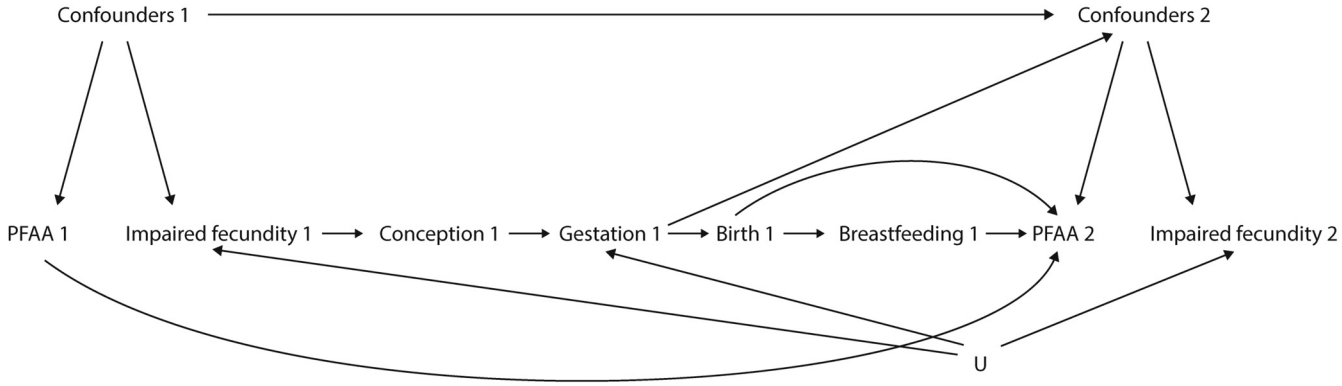

Figure 2. Proposed causal structure of the perfluoroalkyl acids (PFAAs)–time to pregnancy (TTP) association, including time-varying confounding. PFAA 1, TTP 1, and confounders 1 relate to nulliparous women, while PFAA 2, TTP 2, and confounders 2 relate to parous women. Important confounders of the association between PFAAs and the TTP may include, e.g., age, body mass index (BMI), and socioeconomic status. Note: U, unmeasured factors.

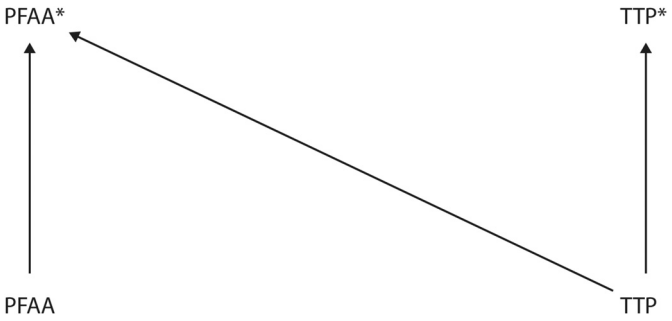

Figure 3. Reverse causation as described by Olsen et al. (2009), Vestergaard et al. (2012), and Whitworth et al. (2012): differential measurement error of perfluoroalkyl acids (PFAAs) measured during pregnancy in parous women (indicated by PFAA*) as a proxy for PFAA concentrations during the etiologically relevant time window [during the time period of attempted conception (indicated by PFAA)]. TTP* designates the recorded TTP.

during the causal time window (prior to conception). However, after a decline following the previous pregnancy, childbirth, and breastfeeding, PFAA concentrations in parous women may increase, and the longer the time to a subsequent pregnancy, the higher the concentrations measured during the next pregnancy may become (Papadopoulou et al. 2015; Whitworth et al. 2012). An increase in PFAA concentrations measured during the next pregnancy may thus be proportional to the interpregnancy interval, including but not limited to the TTP. Thus, this issue may be described as dependent measurement error (VanderWeele and Hernán 2012) (Figure 4), since the levels of PFAAs and the TTP are connected through a common cause rather than through a direct arrow from the TTP to the measured PFAA levels. Such common causes may include any unmeasured factors that determine fecundability in both pregnancies, e.g., genetic factors or time-stable environmental factors. Of note, a study of associations between perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) and the TTP in a pooled sample of parous and nulliparous women who were planning to become pregnant, which measured PFAA concentrations during the causal time window (prior to conception), reported no association (Buck Louis et al. 2013). In contrast, some pregnancy-based studies reported associations between PFAAs measured during pregnancy and the TTP in parous women or in pooled samples of parous and nulliparous women (Bach et al. 2015b; Vélez et al. 2015; Whitworth et al. 2012). For a complete overview of the existing literature, please refer to our systematic review on this topic (Bach et al. 2016b).

Conditioning on previous childbirths and the interpregnancy interval would theoretically remove the risk of information bias caused by these two factors in pregnancy-based studies, but according to Figure 2, additional adjustment is necessary in order to achieve estimates unbiased by confounding. Figure 5 illustrates the issue of potential dependent measurement error (Figure 4) within the context of Figure 2.

If previous PFAA concentrations or confounders of PFAA–TTP associations in previous pregnancies are not included in the statistical model for parous women (in addition to common causes of current PFAA concentrations and the TTP and data on previous births), we hypothesized that PFAAs might be associated with the TTP even if a causal association does not exist, regardless of whether PFAAs were measured before conception or during pregnancy. Further, in pregnancy-based cohorts, we hypothesized that not accounting for the interpregnancy interval in parous women would also result in an association between PFAAs and longer TTP, even if a causal association does not exist. However, if the study population is restricted to nulliparous women, only the confounders of the index pregnancy would need to be considered in order to achieve unbiased estimates. Thus, we expected that if no causal association exists between preconception PFAAs (during the causal time window) and fecundability (as indicated by the TTP), statistical associations between PFAAs and the TTP would be present in parous women (i.e., PFAA 2 would be associated with impaired fecundity 2), but not in nulliparous women (i.e., PFAA 1 would not be associated with impaired fecundity 1). We evaluated this hypothesis in a sample of women from the Danish National Birth Cohort with measurements of PFAA concentrations during early pregnancy, estimating the association between PFAA levels and the TTP separately for parous and nulliparous women. To account for potential exposure measurement error in parous women, we also aimed to estimate this association accounting for the interpregnancy interval in addition to potential confounders.

Methods

Study Population

We studied a sample of 1,251 women who participated in the Lifestyle During Pregnancy Study nested in the Danish National

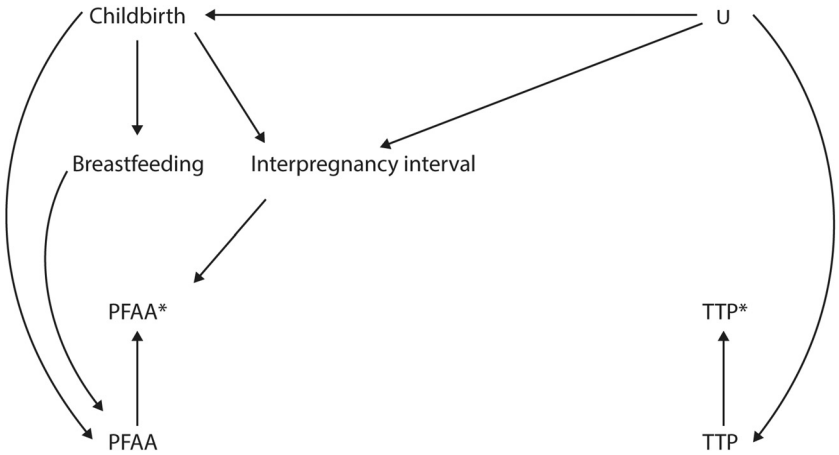

Figure 4. Dependent measurement error of perfluoroalkyl acids (PFAAs) measured during pregnancy in parous women. PFAA* designates PFAA concentrations measured during pregnancy, a proxy for PFAA concentrations during the etiologically relevant time window [during the time period of attempted conception (indicated by PFAA)]. TTP* designates the recorded time to pregnancy (TTP). Unmeasured common causes (U) of the TTP for the current pregnancy, the interpregnancy interval, and previous pregnancies and births may include e.g. genetic or time-stable environmental factors.

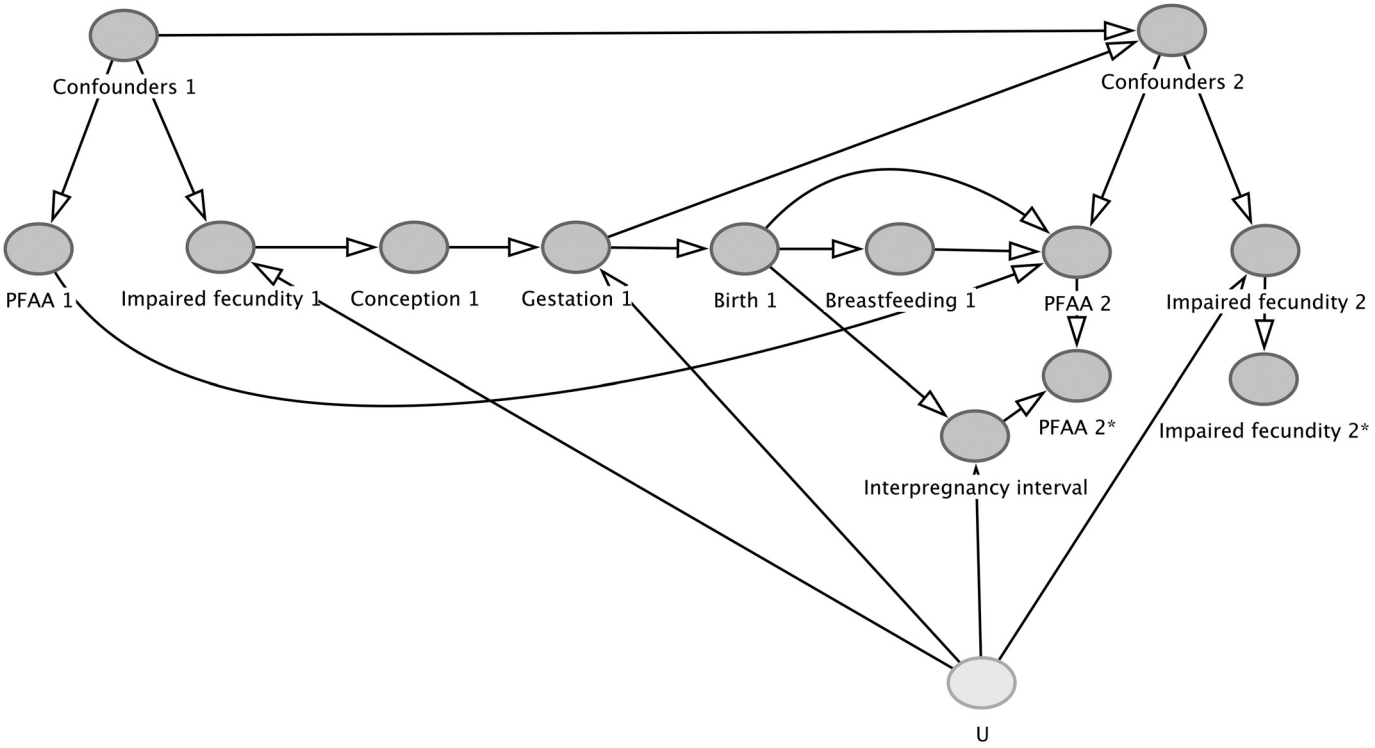

Figure 5. Proposed causal structure of the perfluoroalkyl acid (PFAA)–time to pregnancy (TTP) association, combining Figures 2 and 3 to simultaneously illustrate confounding and dependent measurement error of PFAA levels. PFAA 1, TTP 1, and confounders 1 relate to nulliparous women, while PFAA 2, TTP 2, and confounders 2 relate to parous women. PFAA* designates the PFAA level measured during pregnancy. Important confounders of the association between PFAAs and the TTP may include, e.g., age, BMI, and socioeconomic status. Note: U, unmeasured factors. Figure made by use of www.daggity.net.

Birth Cohort (1996–2002), had plasma PFAA concentrations measured in early pregnancy (median: 8 wk; interquartile range: 7–10 wk), had a planned pregnancy, and had information on the TTP. Because the primary objective of the Lifestyle During Pregnancy Study (Kesmodel et al. 2010) was to examine the association between lifestyle during pregnancy and offspring neurodevelopment, participants were sampled from the Danish National Birth Cohort according to maternal alcohol consumption patterns. The sample of women included in the present analysis has not previously been investigated regarding the association between PFAAs and the TTP. The study was approved by the Danish Data Protection Agency (reference 2012-41-1288) and the Danish National Committee on Health Research Ethics (reference M-20110054). All participants gave informed consent at the time they entered the Danish National Birth Cohort.

Exposure Assessment

PFAAs were measured in plasma by high-performance liquid chromatography–tandem mass spectrometry after solid phase extraction. All samples were analyzed at the Department of Environmental Science, Aarhus University, Roskilde, Denmark. Contrary to the previous studies in the Danish National Birth Cohort on the association between PFAAs and the TTP (Bach et al. 2015b; Fei et al. 2009), we included all PFAAs quantifiable in at least 75% of the samples, including PFOS, PFOA, perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA). PFAA concentrations were available for only one pregnancy per woman in the present study sample. The transportation, processing, and storage of the samples did not differ according to parity. The limits of quantification (LOQ), the numbers and proportions of samples below the LOQ for each PFAA, and the precision of the method are presented in Table S1.

Statistical Analyses

In this scenario, we relied on the strong assumptions of no selection bias (which might have resulted from restricting the cohort to women with planned pregnancies who had a live birth, and from excluding infertile couples who did not conceive), no outcome measurement error, and no unmeasured confounding (other than unmeasured confounding indicated by U in Figures 2, 4, and 5).

To evaluate the influence of parity on the association between PFAAs and the TTP, we applied separate discrete time survival models to estimate fecundability ratios (FRs) according to plasma PFAA quartiles for parous and nulliparous women, respectively. Analyses of the TTP were censored after 12 mo. PFAA concentrations below the LOQ were replaced with the LOQ divided by two (Helsel 1990; Hornung and Reed 1990). PFAA quartiles were defined separately for nulliparous and parous women, since all analyses were stratified by parity. The analyses were conducted as described in further detail in Bach et al. (2015b) and adjusted for age (continuous), socioeconomic status (three groups according to maternal education and job status; if this information was missing, the corresponding paternal information was used), and prepregnancy BMI (continuous) (Model A). In all analyses, we applied inverse probability weighting of the sampling fractions, using robust standard errors, to take into account the sampling of the participants from the baseline cohort.

To examine whether the variance in the PFAA concentrations might be explained by differences in the interpregnancy interval, we estimated unadjusted Spearman's correlations between PFAA concentrations and the interpregnancy interval. Further, to illustrate the importance of accounting for the interpregnancy interval, we estimated associations between interpregnancy intervals and PFAA concentrations in the index pregnancy using restricted cubic splines (5 knots). To account for potential exposure measurement error in parous women, we repeated the

primary analyses using two different approaches to account for the interpregnancy interval. First (Model B), we additionally adjusted for the interpregnancy interval, i.e., the time from the birth of a child to the next conception (restricted cubic spline). A statistical concern regarding this analysis was the potentially high covariance between the TTP and the interpregnancy interval. To examine possible associations between the TTP and the interpregnancy interval, we estimated unadjusted Spearman's correlations between the TTP and the interpregnancy interval in the parous women. Second (Model C), we applied a modified version of a previously proposed approach to correct for sampling conditions affecting biomarker concentrations (Mortamais et al. 2012), first modeling the unadjusted association between the interpregnancy interval and each PFAA, taking into account the nonmonotonic relationship using a restricted cubic spline (linear regression). From this model, we predicted a PFAA level for each woman. Using the predicted PFAA concentrations, we calculated a correction factor corresponding to the difference between the predicted value for each woman and the predicted PFAA level at the median of the interpregnancy interval (2.6 y). Finally, we generated corrected PFAA concentrations by subtracting the correction factor from the measured PFAA concentrations and generated new PFAA quartiles using these corrected PFAA concentrations. This exposure measure was then used to estimate FRs adjusted for the same covariates as Model A.

We conducted further analyses to illustrate some of the basic assumptions in Figure 2. First, we carried out a sensitivity analysis for unmeasured confounding in parous women. Assuming various prevalences of an unmeasured confounder in women with exposure levels in the highest and lowest exposure quartile of PFOS, we estimated the strength of the confounder–outcome association needed to bias a true null association between PFOS and TTP to an estimated FR of 0.70 (Schneeweiss 2006).

Finally, we conducted additional sensitivity analyses using identical quartile cutpoints for nulliparous and parous women, and censoring at 6 mo instead of 12 mo.

The statistical analyses were performed in Stata (version 13; StataCorp).

Results

PFOS and PFOA were quantified in all samples. PFNA and PFDA had the largest proportions of samples below the LOQ, with more samples below the LOQ for parous women (in parous women, the highest proportion below the LOQ was 7.5% for PFNA). Participant characteristics according to quartiles of PFAAs are shown in Table 1. LOQ are shown in Table S1, and quartile definitions for each PFAA are shown in Table 1. There was no clear association between PFAA quartiles and age, BMI, or socioeconomic status.

Associations between Quartiles of Perfluoroalkyl Acids and the Time to Pregnancy according to Parity

In general, estimated FRs were close to the null for PFAA quartiles and the TTP in nulliparous women (Model A) (Table 2). In parous women, FRs were <1 for quartiles 2–4 relative to the lowest quartile, indicating longer TTP (see Table 2). The strongest associations were estimated for PFHxS and PFOS [FR = 0.60; 95% confidence interval (CI): 0.45, 0.80 and FR = 0.60; 95% CI: 0.44, 0.82 for the highest vs. lowest quartiles, Model A]. Corresponding FRs for PFHpS and PFOA were similar in magnitude, while FRs for PFNA and PFDA were closer to the null.

Associations between Quartiles of Perfluoroalkyl Acids and the Time to Pregnancy Correcting for the Interpregnancy Interval

A kernel density plot of the interpregnancy interval is provided in Figure S1, illustrating that for the included women who had a second pregnancy, most of them became pregnant again within 5 y with a peak at approximately 2.5 y. Parous women classified in the highest quartiles of plasma PFHxS, PFHpS, PFOS, and PFOA had longer interpregnancy intervals than women in lower quartiles, while interpregnancy intervals were longer for women in the two highest vs. two lowest quartiles of PFNA and PFDA (Table 1). Correlations (r_s) between the interpregnancy interval and PFAA concentrations were 0.13, 0.32, 0.19, 0.10, 0.13, and 0.10 for PFOS, PFOA, PFHxS, PFHpS, PFNA, and PFDA, respectively. When associations between PFAA concentrations and the interpregnancy interval were modeled using restricted cubic splines, PFHXS, PFNA, and PFDA did not vary during the first 1 to 2 y following a previous pregnancy and then increased up until a second plateau at 3–4 y (Figure 6). For PFHpS, PFOS, and PFOA, concentrations decreased during the first 1.5 to 2 y and then increased. For PFOA, concentrations continued to increase through the 6-y follow-up period, while PFHpS and PFOS began to decrease slightly approximately ~3–4 y after a previous pregnancy.

In general, estimated FRs for parous women were closer to null after further adjustment for the interpregnancy interval (Model B) (Table 2). The percentage difference from Model A to Model B for FRs comparing the highest vs. lowest quartile ranged from 10% (95% CI: −2, 23%) for PFDA to 24% (95% CI: 6, 46%) for PFOA. The correlation between the TTP and the interpregnancy interval was low ($r_s = 0.18$), and the interpregnancy interval explained only 3% of the variation in the TTP (squared $r_s = 0.03$), indicating that potential collinearity is not a major concern in analyses conditioning on the interpregnancy interval.

Estimates for parous women from models using PFAA quartiles corrected to the median interpregnancy interval (Model C) also moved closer to the null, similar to estimates from the models adjusted for the interpregnancy interval (Model B) (Table 2). For PFHxS, PFHpS, PFOS, and PFOA, Model C estimates were even closer to the null than estimates from Model B. Compared with Model A, Model C FRs for the highest vs. lowest quartile increased from 11% (95% CI: −2, 26%) for PFDA to 40% (95% CI: 11, 76%) for PFOS. PFAA distributions changed after correction for the interpregnancy interval [specifically, after subtracting the difference between the predicted value for each woman and the predicted PFAA level at the median of the interpregnancy interval (2.6 y) from the measured PFAA concentration for each woman], resulting in new quartile cutpoints and reclassification of PFAA quartiles for 14% (for PFDA) to 33% (for PFOA) of parous women relative to the main analyses (Table S2).

Potential Impact of Unmeasured Confounding

Table 3 shows results of a sensitivity analysis to determine the conditions required for an unmeasured dichotomous confounder to bias a true null association between PFOS and TTP to an estimated FR of 0.70 (approximately equal to the adjusted Model B FR of 0.69 for the highest vs. lowest quartile of PFOS in Table 2.) For example, if the prevalence of the confounder was 0.9 and 0.1 among women in the highest and lowest PFOS quartiles, respectively, the risk ratio for the confounder–fecundability association would have to be 0.65 to bias a true null association from 1.0 to 0.70, while an unmeasured dichotomous confounder with a weaker association with exposure would require a stronger association

Table 1. Participant characteristics according to quartiles of plasma perfluoroalkyl acids measured in early pregnancy (median 8 wk of gestation) in nulliparous and parous women, respectively, from the Danish National Birth Cohort, 1996–2002.

Nulliparous women ($n = 638$)						Parous women ($n = 613$)						
PFAA (ng/mL)	Age (years)	Prepregnancy BMI (kg/m^2)	Socioeconomic status (column %)			PFAA (ng/mL)	Age (years)	Prepregnancy BMI (kg/m^2)	Socioeconomic status (column %)			Interpregnancy interval (days)
—	Median (IQR)	Median (IQR)	Low $n = 33$	Middle $n = 191$	High $n = 414$	—	Median (IQR)	Median (IQR)	Low $n = 56$	Middle $n = 205$	High $n = 352$	Median (IQR)
PFHxS	—	—	—	—	—	PFHxS	—	—	—	—	—	—
0.27–0.91	28 (26–31)	22 (21–25)	30	25	23	<0.08–0.65[a]	32 (29–35)	23 (21–25)	23	22	24	811 (490–1,204)
0.92–1.22	28 (26–30)	23 (21–25)	15	28	24	0.66–0.92	32 (29–34)	23 (21–26)	27	26	25	841 (533–1,333)
1.23–1.53	29 (27–31)	23 (21–26)	21	21	27	0.93–1.20	32 (30–35)	23 (21–26)	27	28	24	1,044 (607–1,646)
1.54–12.80	29 (27–31)	23 (21–24)	33	26	26	1.21–5.39	32 (29–35)	23 (21–25)	23	24	27	1,094 (759–1,743)
PFHpS	—	—	—	—	—	PFHpS	—	—	—	—	—	—
<0.11–0.32[a]	29 (26–31)	22 (20–24)	27	21	29	<0.11–0.24[a]	33 (30–36)	22 (21–24)	21	17	27	769 (500–1,204)
0.33–0.41	29 (26–31)	23 (21–25)	21	22	24	0.25–0.32	31 (29–34)	23 (21–25)	23	28	23	916 (596–1,443)
0.42–0.54	28 (26–31)	23 (21–25)	18	27	24	0.33–0.42	32 (30–35)	23 (21–26)	25	27	26	949 (560–1,592)
0.55–1.73	29 (26–31)	23 (21–26)	33	30	22	0.43–2.01	32 (29–34)	23 (21–27)	30	27	24	1,073 (714–1,663)
PFOS	—	—	—	—	—	PFOS	—	—	—	—	—	—
6.7–23.4	29 (26–31)	22 (20–24)	27	19	27	6.3–20.7	33 (29–36)	22 (21–24)	23	18	24	828 (517–1,224)
23.5–30.2	29 (26–32)	22 (20–25)	27	25	26	20.8–26.0	32 (30–34)	23 (21–25)	18	25	27	843 (534–1,592)
30.3–38.0	29 (26–31)	23 (21–25)	6	24	25	26.1–33.6	32 (29–35)	23 (21–26)	27	29	24	977 (579–1,492)
38.1–117.0	27 (26–30)	23 (21–26)	39	32	22	33.7–127.0	32 (29–34)	23 (21–26)	32	28	25	1,126 (755–1,651)
PFOA	—	—	—	—	—	PFOA	—	—	—	—	—	—
1.21–4.02	29 (26–32)	23 (20–25)	24	26	26	0.61–2.60	32 (30–35)	22 (21–25)	20	15	26	627 (390–977)
4.03–5.03	29 (26–31)	22 (21–25)	21	23	23	2.61–3.43	32 (29–35)	23 (21–26)	38	25	25	895 (569–1,358)
5.04–6.13	28 (25–30)	23 (21–26)	24	26	24	3.44–4.53	32 (29–34)	23 (21–26)	18	30	23	973 (629–1,451)
6.14–13.80	29 (26–31)	23 (21–25)	30	26	27	4.54–15.00	32 (30–35)	23 (21–25)	25	30	26	1,268 (822–2,060)
PFNA	—	—	—	—	—	PFNA	—	—	—	—	—	—
<0.27–0.39[a]	28 (26–31)	22 (20–24)	30	21	27	<0.27–0.35[a]	32 (29–35)	23 (21–26)	20	27	25	824 (484–1,229)
0.40–0.48	28 (26–31)	23 (21–26)	27	24	23	0.36–0.43	32 (29–35)	23 (22–25)	27	29	22	895 (563–1,503)
0.49–0.61	28 (26–30)	23 (21–26)	24	31	23	0.44–0.54	32 (29–34)	23 (21–26)	34	21	23	1,051 (679–1,590)
0.62–2.23	29 (27–32)	23 (21–25)	18	24	27	0.55–2.16	32 (30–35)	23 (21–25)	20	23	30	1,029 (656–1,610)
PFDA	—	—	—	—	—	PFDA	—	—	—	—	—	—
<0.09–0.14[a]	28 (25–30)	23 (21–25)	33	30	31	<0.09–0.13[a]	31 (28–34)	23 (21–26)	25	30	20	826 (497–1,289)
0.15–0.17	29 (26–31)	23 (20–25)	15	21	17	0.14–0.17	32 (29–34)	23 (21–25)	25	27	27	904 (521–1,391)
0.18–0.22	29 (27–31)	23 (21–26)	30	23	27	0.18–0.22	33 (30–35)	23 (21–26)	23	23	27	1,092 (639–1,702)
0.23–0.87	29 (27–31)	23 (21–25)	21	26	25	0.23–0.90	32 (30–35)	23 (21–25)	27	20	27	982 (658–1,513)

Note: —, no data; BMI, body mass index; CI, confidence interval; IQR, interquartile range; LOQ, limit of quantification; PFAA, perfluoroalkyl acid; PFHpS, perfluoroheptane sulfonate; PFHxS, perfluorohexane sulfonate; PFDA, perfluorodecanoic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate.

[a]Numbers and proportions of women with PFAA values below the LOQ: PFHxS: nulliparous = 0 (0%), parous = 1 (0.2%); PFHpS: nulliparous 1 (0.2%), parous = 5 (0.8%); PFNA: nulliparous = 22 (3.3%), parous = 47 (7.5%); PFDA: nulliparous = 17 (2.3%), parous = 25 (4.0%). Values <LOQ were replaced as LOQ/2.

Table 2. Fecundability ratios according to quartiles of perfluoroalkyl acids in 638 nulliparous and 613 parous women from the Danish National Birth Cohort, 1996–2002.

—	Nulliparous ($n = 638$)	Parous ($n = 613$)	Parous ($n = 604$)	—	—	—
PFAA quartile	Model A: adjusted FR (95% CI)	Model A: adjusted FR (95% CI)	Model B: adjusted FR (95% CI)	Percent change in FR (95% CI) comparing Model B to A	Model C: adjusted FR (95% CI)	Percent change in FR (95% CI) comparing Model C to A
PFHxS	—	—	—	—	—	—
1	1.00 (reference)	1.00 (reference)	1.00 (reference)	—	1.00 (reference)	—
2	1.03 (0.81–1.32)	0.74 (0.55–1.01)	0.80 (0.59–1.08)	7 (−4, 19)	1.08 (0.79–1.49)	46 (4, 103)
3	1.06 (0.83–1.35)	0.79 (0.59–1.04)	0.88 (0.65–1.19)	12 (0, 25)	0.94 (0.71–1.26)	20 (−4 50)
4	0.92 (0.72–1.18)	0.60 (0.45–0.80)	0.71 (0.53–0.94)	18 (5, 32)	0.82 (0.62–1.08)	36 (12, 65)
PFHpS	—	—	—	—	—	—
1	1.00 (reference)	1.00 (reference)	1.00 (reference)	—	1.00 (reference)	—
2	0.89 (0.71–1.11)	0.78 (0.57–1.07)	0.84 (0.62–1.15)	8 (−3, 20)	1.05 (0.77–1.44)	35 (−6, 94)
3	0.86 (0.68–1.09)	0.80 (0.59–1.09)	0.86 (0.63–1.19)	7 (−5, 21)	1.07 (0.78–1.49)	34 (5, 72)
4	1.04 (0.81–1.33)	0.67 (0.49–0.93)	0.79 (0.58–1.09)	18 (5, 32)	0.87 (0.63–1.20)	29 (5, 59)
PFOS	—	—	—	—	—	—
1	1.00 (reference)	1.00 (reference)	1.00 (reference)	—	1.00 (reference)	—
2	0.81 (0.64–1.03)	0.55 (0.41–0.76)	0.60 (0.58–0.63)	9 (−4, 23)	0.85 (0.62–1.16)	53 (9, 116)
3	0.90 (0.70–1.17)	0.69 (0.51–0.94)	0.73 (0.70–0.76)	6 (−5, 17)	0.92 (0.67–1.27)	34 (3, 74)
4	0.89 (0.69–1.15)	0.60 (0.44–0.82)	0.69 (0.66–0.73)	16 (1, 33)	0.84 (0.61–1.15)	40 (11, 76)
PFOA	—	—	—	—	—	—
1	1.00 (reference)	1.00 (reference)	1.00 (reference)	—	1.00 (reference)	
2	0.98 (0.76–1.27)	0.76 (0.56–1.03)	0.82 (0.79–0.86)	9 (−4, 23)	0.97 (0.72–1.32)	28 (−15, 93)
3	1.01 (0.79–1.30)	0.84 (0.61–1.14)	1.00 (0.96–1.05)	20 (6, 35)	0.88 (0.65–1.21)	6 (−23, 45)
4	0.92 (0.73–1.15)	0.63 (0.47–0.86)	0.79 (0.75–0.83)	24 (6, 46)	0.85 (0.62–1.16)	34 (5, 70)
PFNA	—	—	—	—	—	—
1	1.00 (reference)	1.00 (reference)	1.00 (reference)	—	1.00 (reference)	—
2	0.90 (0.72–1.13)	0.86 (0.64–1.17)	0.92 (0.88–0.96)	7 (6, 22)	1.08 (0.80–1.46)	25 (−9, 73)
3	1.12 (0.88–1.43)	0.87 (0.65–1.16)	0.99 (0.95–1.03)	14 (0, 29)	1.07 (0.80–1.45)	24 (3, 49)
4	0.93 (0.74–1.17)	0.86 (0.64–1.15)	0.97 (0.93–1.01)	13 (0, 28)	0.96 (0.71–1.29)	12 (−3, 29)
PFDA	—	—	—	—	—	—
1	1.00 (reference)	1.00 (reference)	1.00 (reference)	—	1.00 (reference)	—
2	1.13 (0.89–1.43)	0.92 (0.67–1.27)	0.99 (0.95–1.04)	8 (−6, 23)	0.92 (0.68–1.26)	0 (−24, 31)
3	1.02 (0.82–1.28)	0.87 (0.65–1.16)	1.02 (0.98–1.07)	18 (4, 33)	0.95 (0.71–1.28)	10 (−12, 36)
4	1.11 (0.89–1.39)	0.78 (0.58–1.03)	0.85 (0.82–0.89)	10 (−2, 23)	0.86 (0.65–1.15)	11 (−2, 26)

Note: —, no data; CI, 95% confidence interval; FR, fecundability ratio; PFAA, perfluoroalkyl acid; PFDA, perfluorodecanoic acid; PFHpS, perfluoroheptane sulfonate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate.
Model A: Adjusted for age, socioeconomic status, and prepregnancy body mass index.
Model B: Model A additionally adjusted for the interpregnancy interval.
Model C: Model A, using PFAA quartiles corrected to an interpregnancy interval of median length (2.6 y).
PFAA quartile cutpoints for Models A and B are provided in Tables 1 and 2 for nulliparous and parous women, respectively. Quartile cutpoints for Model C are shown in Table S2.

with TTP, e.g., relative risk (RR) = 0.05 for a confounder with a prevalence of 0.6 and 0.4 in the highest and lowest PFOS quartiles, respectively.

Additional Sensitivity Analyses

In the sensitivity analysis using identical rather than parity-specific PFAA quartiles (Model A), associations in parous women remained inverse but were slightly attenuated for PFHxS, PFHpS, PFOS, and PFOA (Table S3). In the nulliparous women, FRs remained close to the null, with the exception of the PFHxS, where the inverse association for the highest vs. lowest quartile increased from 0.92 (95% CI: 0.72, 1.18) to 0.80 (95% CI: 0.61, 1.04). The results of the sensitivity analysis censoring at 6 mo were similar to those of the primary analysis for the nulliparous women and slightly attenuated for the parous women (Table S4).

Discussion

In a previously unstudied sample of women from the Danish National Birth Cohort, including a large number of nulliparous women, we found no clear association between PFAAs and the TTP (adjusting for age, socioeconomic status, and prepregnancy BMI) in nulliparous women. In parous women, using the same statistical model, higher PFAA quartiles were, however, consistently associated with longer TTP, with the lowest FRs estimated for women in the highest PFAA quartiles, except for PFOS. According to our proposed causal structure, confounding induced by factors related to previous pregnancies is a plausible explanation for the difference in findings for parous and nulliparous women. Consistent with this model, associations in parous women were attenuated when we used PFAA quartiles corrected to the median interpregnancy interval and when we adjusted for the interpregnancy interval (which was not associated with the TTP), which suggests that when PFAA concentrations are measured in samples collected during pregnancy rather than during the time when women are trying to become pregnant, exposure measurement error may also play a role. However, PFAAs still tended to be associated with a longer TTP in parous women, despite the use of statistical methods to account for the interpregnancy interval. Given the null association in nulliparous women, we hypothesize that the association in parous women was due to residual confounding. However, the hypothesis of an effect of the exposure on fecundability, with a bias towards attenuation that would be more pronounced in nulliparous compared to parous women, cannot be excluded.

We did not have information on confounders or PFAA concentrations for the previous pregnancy in parous women, and were only able to examine the potential impact of uncontrolled confounding in a simple sensitivity analysis that assumed that the influence of all unmeasured or unknown potential confounders could be represented by a single dichotomous confounder. Based on this analysis, an unmeasured confounder with a prevalence of 0.7 among women with the highest PFOS quartile and 0.3 among women in the lowest PFOS quartile would have to have an RR of

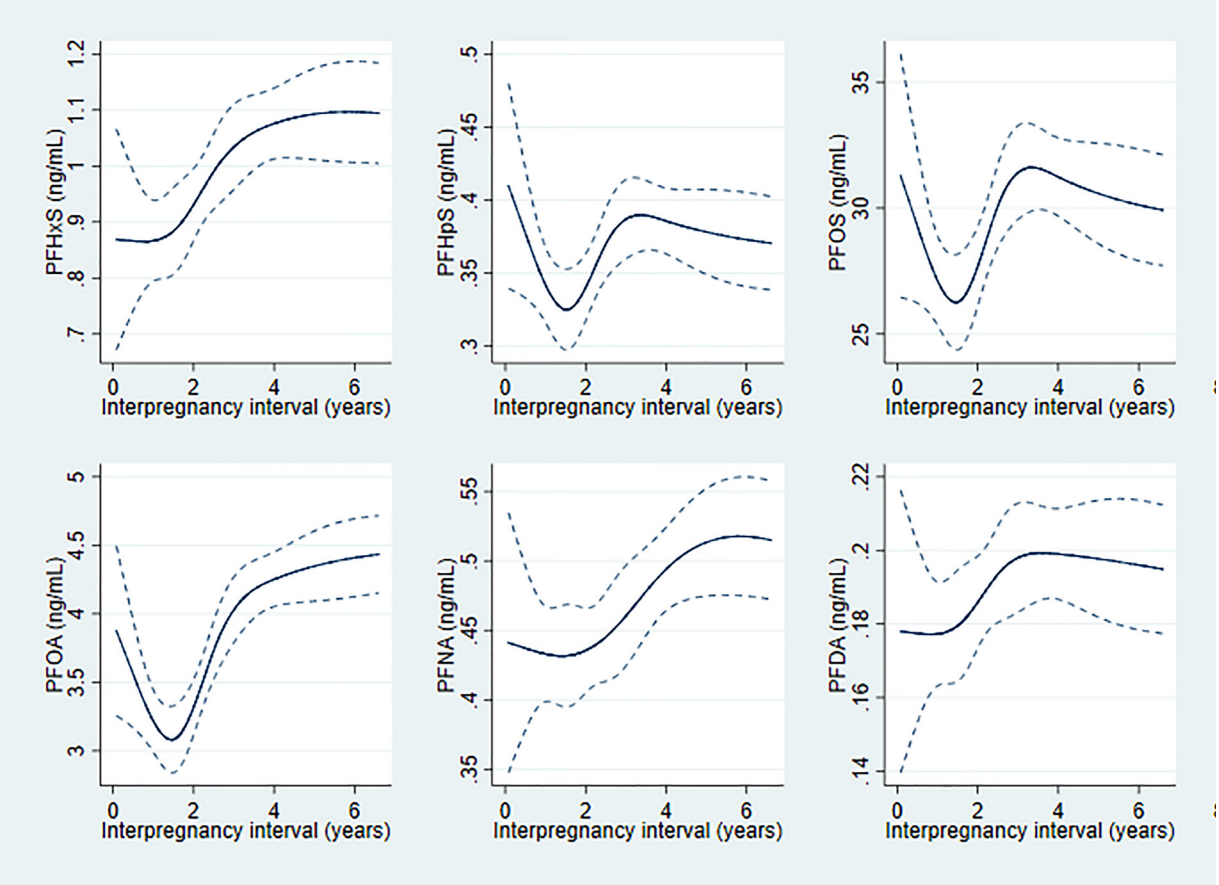

Figure 6. The association between the interpregnancy interval and perfluoroalkyl acid (PFAA) concentrations in the current pregnancy in 604 parous women from the Danish National Birth Cohort, 1996–2002. PFAA concentrations were measured at a median of 8 wk of gestation. The interpregnancy interval was modeled using restricted cubic splines (5 knots). The figures are restricted to women with an interpregnancy interval <6 y and 7 mo (90th percentile). The solid lines illustrate the estimated regression coefficients, and the dashed lines indicate the upper and lower 95% confidence intervals (CIs).

0.40 for the association between the confounder and fecundability for uncontrolled confounding to bias a true null association to an FR of 0.70 for PFOS (approximately equal to the Model B FR for the highest vs. lowest quartile). Whereas this may be considered an example of relatively strong confounding by a single factor, it may be feasible for multiple unmeasured or unknown confounders to exert an impact of this magnitude. We consider that unmeasured and unknown common causes of impaired fecundability in consecutive pregnancies (U in Figure 2) including, e.g., genetic causes or tubal dysfunction may impact the measured associations between PFAA levels and impaired fecundability, as illustrated in Figure 2, through open backdoor paths, including several variables (see Figure 2, e.g., through PFAA 1, Confounders 1, Impaired fecundity 1, U).

Consequently, if PFAAs are measured during pregnancy and the proposed causal structure is true, confounding by PFAA levels or confounders of the PFAA–TTP association for the previous pregnancy may account for most of the estimated associations

Table 3. Confounder–outcome risk ratios required for uncontrolled confounding by a single dichotomous confounder to bias a true null association between perfluorooctane sulfonate (PFOS) and time to pregnancy (TTP) among parous women to fecundability ratio (FR) = 0.70, according to the prevalence of the confounder among women in the highest and lowest PFOS quartiles.

Prevalence in highest quartile	Prevalence in lowest quartile								
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0.9	0.65	0.60	0.55	0.50	0.45	0.40	0.25	0.10	—
0.8	0.60	0.55	0.50	0.40	0.35	0.20	0.05	—	—
0.7	0.50	0.45	0.40	0.30	0.15	—	—	—	—
0.6	0.45	0.35	0.25	0.05	—	—	—	—	11.01
0.5	0.30	0.15	—	—	—	—	—	6.01	3.30
0.4	0.10	—	—	—	—	16.02	4.35	2.85	2.30
0.3	—	—	—	—	7.01	3.50	2.60	2.15	1.90
0.2	—	—	30.98	4.75	3.00	2.35	2.05	1.85	1.70
0.1	—	8.51	3.70	2.65	2.20	1.95	1.75	1.65	1.55

Note: —, no data.
Values shown in the body of the table are risk ratios for the confounder–outcome association required, given the corresponding prevalences among women in the highest and lowest PFOS quartiles, for an FR = 0.70 to have been entirely due to uncontrolled confounding by a single dichotomous confounder. For example, if the prevalence was 0.9 and 0.1 among women in the highest and lowest PFOS quartiles, respectively, the risk ratio for the confounder–TTP association would have to be 0.65 in order to bias a true null association for the highest vs. lowest PFOS quartile and fecundability from 1.0 to 0.70.

between PFAAs and the TTP in parous women. The lack of an association in nulliparous women, which was robust to the use of both identical and separate quartile cutpoints for nulliparous and parous women, and the fact that the majority of previous studies in nulliparous women have reported no association (Bach et al. 2015a; Vestergaard et al. 2012; Whitworth et al. 2016) (further discussed below) suggests that there may not be a causal association between maternal PFAA exposures and the TTP. However, in a pregnancy-based design, exposure misclassification may arguably also play a role in nulliparous women, and based on the current results, it is not possible to completely exclude an effect of PFAAs on the TTP.

Of note, the influence of potential biases may differ according to the type of PFAA. For example, accounting for the interpregnancy interval may be particularly important for PFOA, which had the highest correlation with the interpregnancy interval in our study population (Spearman's $r_s = 0.32$, compared with 0.13 for PFOS). Whitworth et al. (2012) also found the interpregnancy interval to be a stronger predictor of PFOA than PFOS concentrations. In our study, the association between PFOA and TTP also showed the greatest attenuation when adjusted for the interpregnancy interval.

In other studies of the association between PFAAs and TTP in parous women or women of all parity, the majority of studies found associations between in particular PFOS and PFOA and longer TTP. However, only one study demonstrated such an association in nulliparous women (Fei et al. 2012). In the study by Fei et al. (2012) from the Danish National Birth Cohort, the average PFAA concentrations were high compared to studies conducted in other countries as well as during different time periods in Denmark, but similar to those in the present study using a different sample of women from the cohort during the same time period. Studies conducted in Denmark and Norway with *a priori* restriction to nulliparous women found point estimates close to the null for associations between PFAAs and the TTP (Bach et al. 2015a; Vestergaard et al. 2012; Whitworth et al. 2016). We argue that previous estimates of associations between PFAAs and the TTP based on populations that included parous women may have been biased.

Dependent measurement error caused by previous childbirths and the interpregnancy interval could theoretically be alleviated by using a pregnancy planner design (with exposure assessed when women first begin trying to conceive) or by conditioning on the interpregnancy interval and parity in pregnancy-based cohorts (with exposures measured during pregnancy). Moreover, it may also be possible to correct exposure estimates for parous women using toxicokinetic modeling or the simple two-step standardization approach based on regression residuals used in the present study. However, the latter approach needs further investigation, including validation of the two-step standardization for exposures modeled as categorical variables (Mortamais et al. 2012).

Like pregnancy-based studies, pregnancy planner studies that include parous women may be biased by confounders or PFAA concentrations related to the previous pregnancy, in addition to confounding by common causes of current PFAA concentrations and the TTP and previous births. If it is not possible to account for all these issues, it may be preferable to restrict studies to nulliparous women, since only the confounders of the index pregnancy would need to be considered in order to achieve unbiased estimates. However, excluding parous women will limit the generalizability and may complicate interpretation (Sallmén et al. 2015). In contrast with pregnancy-based cohorts, pregnancy planner studies do not exclude infertile women and pregnancies that do not result in live births. However, these exclusions are not sufficient to explain the lack of associations between preconception PFAAs and the TTP in pregnancy planner studies (Buck Louis et al. 2013; Vestergaard et al. 2012). A few studies have investigated associations between PFAAs and the risk of miscarriage. Darrow et al. (2014) found an association between PFOS and miscarriage, which was not corroborated by Jensen et al. (2015). However, Jensen et al. found an association between PFNA and PFDA and miscarriage. None of the studies found associations between PFOA and the risk of miscarriage (Darrow et al. 2014; Jensen et al. 2015; Savitz et al. 2012). An effect of PFAAs on the risk of miscarriage, particularly among women with the highest exposures, might bias associations between PFAAs and the TTP toward the null, especially if PFAAs are measured during pregnancy.

Conclusions

Previously reported associations between PFAAs and the TTP in parous women may be due to confounding by factors related to previous pregnancies and to exposure measurement error caused by the decrease in PFAA concentrations during pregnancy and breastfeeding and the subsequent increase in PFAAs resulting from accumulation during the interpregnancy interval. Consequently, when parous women are included in studies of associations between PFAAs and the TTP, the analyses should be conditioned on: *a*) common causes of PFAA concentrations and the TTP in the current pregnancy, *b*) previous births (a descendant of a collider), *c*) either PFAA concentrations or common causes of PFAA concentrations and the TTP in the previous pregnancy (to alleviate the collider stratification bias introduced by conditioning on a descendant of a collider), and for pregnancy-based birth cohorts studies, and *d*) the interpregnancy interval. An alternative is to restrict studies to nulliparous women, but this may limit generalizability and may cause other problems with interpretation. If relevant information is available, it may also be possible to correct exposure estimates of parous women, e.g., by the use of toxicokinetic modeling. Potential biases related to uncontrolled confounding and measurement error when parous women are included in studies of the TTP may also apply to studies of other chemicals with long biological half-lives that are transferred to the fetus during pregnancy and eliminated via breastmilk.

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